The Senegal DNA Haplotype Is Associated With the Amelioration of Anemia in African-American Sickle Cell Anemia Patients

By Ronald L. Nagel, Soli Eriksesson, Mary E. Fabry, Helena Croizat, Sandra M. Susuka, Herbert Lachman, Milicent Sutton, Catherine Driscoll, Eric Bouhassira, and Henny H. Billett

We have previously determined that in African sickle cell anemia (SS) patients three different β-like globin gene cluster haplotypes are associated with different percent HbF (one of the two types of non-α chains comprising hemoglobin F [HbF]), mean percent HbF, and percent dense cells. We report now that in adult New York SS patients, the presence of at least one chromosome with the Senegal haplotype is associated with higher Hb levels (1.2 g/dL higher) than is found for any other non-Senegal haplotype (P < .004). The percent reticulocytes and the serum bilirubin levels were lower in these patients. When the effect of α-gene number was analyzed by examining a sample of SS patients with concomitant α-thalassemia, the same results were obtained. Because the HbF level is significantly higher among the Senegal haplotype carriers in this sample, the inhibitory effect on sickling of this Hb variant may be one of the reasons for the haplotype effect. We conclude that the Senegal β-like globin gene cluster haplotype is associated with an amelioration of the hemolytic anemia that characterizes sickle cell disease.

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MATERIALS AND METHODS

RESULTS

DNA haplotype composition. The sample consisted of 2 Senegal/Senegal, 4 Senegal/Bantu, 6 Senegal/Benin, 2 Seneg

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0006-4971/91/7706-0011$3.00/0

gal/atypical, 21 Benin/Benin, 2 Bantu/Bantu, 18 Benin/ Bantu, 1 Bantu/atypical, and 4 Benin/atypical. None of the atypicals haplotype had a 5' subhaplotype of the Senegal type nor were XmnI (+).

**Cluster analysis.** A sample of 60 SS patients were subjected to cluster analysis based on the following parameters: haplotype (divided into two classes: one formed by patients with at least one Senegal haplotype and another with patients bearing any other combination of haplotypes), absolute HbF, percent SS4 or dense cells, Hb level, and percent \( ^{5/7} \gamma \). The analysis was allowed to define two clusters, and the results are depicted in Fig 1 using only absolute HbF, Hb, and percent \( ^{5/7} \gamma \) as parameters for the three-dimensional plot, although all parameters are included in the analysis. Notice that all solid circle symbols (cluster 1) are located almost exclusively in the upper portions of the diagram. In a two-dimensional display of the same cluster analysis (Fig 2) using Hb (g/dL) as the abscissa and percent \( ^{5/7} \gamma \) as the ordinate, the separation of two clusters is easily recognized: cluster 1 (solid circles) consisted of 14 members of the sample (23.3%), all of which are patients with at least one Senegal haplotype. The second cluster defined by this analysis constituted 76.6% of the sample or 46 individuals, and when analyzed for haplotype composition it was constituted entirely of patients devoid of Senegal chromosomes.

Because there was an excess of \( \alpha \)-thalassemics in the Senegal group we asked if this parameter could explain the results. A cluster analysis was conducted in the 20 patients that had SS concomitantly with \( \alpha \)-α or \( \alpha \)-α. Figure 3 depicts the cluster analysis based on haplotype (presence or absence of Senegal haplotype), percent SS4 or dense cells, absolute HbF (g/dL), Hb level (g/dL), and percent \( ^{5/7} \gamma \): all seven patients bearing the Senegal haplotype clustered together. This is easily seen on a lateral view (two-dimensional plot) of Fig 3 in which the ordinate is percent \( ^{5/7} \gamma \) and the abscissa is HbF (g/dL) (Fig 4).

**Comparison of the means.** The comparison of the means for several hematologic and blood chemistry parameters are summarized in Table 1 for the 14 patients in cluster 1 (all having at least one chromosome with the Senegal haplotype) and the 46 patients in cluster 2 (all having several combinations of Benin, Bantu, and atypical haplotypes). An important result is that the two groups differ not only in the level of Hb (\( P < .004 \) level) but also in hemolysis-related parameters: (1) total bilirubin and indirect bilirubin levels were significantly lower (\( P < .02 \) to .01); (3) reticulocyte count was also significantly lower (\( P < .01 \)); and in their percent \( ^{5/7} \gamma \), but also haplotype, percent dense cells, and HbF (grams per deciliter).

In addition, these two groups differ in their absolute HbF (0.93 v 0.62, \( P < .03 \)) and in their percent \( ^{5/7} \gamma \) level (60.3% v 46.5%, a difference that is significant at a \( P < 2.2 \times 10^{-14} \)). Patients in cluster 2 had about double the average number of dense cells, and the difference was significant at \( P < .03 \).

To discern the influence of \( \alpha \)-thalassemia on the results outlined above, we compared a subset of patients all exhibiting three or two \( \alpha \)-genes. The results obtained were fundamentally the same; although not all parameters reached significance, the trends were the same as the total sample (Table 2). Noteworthy are the significant differences in Hb (\( P < .003 \)), percent \( ^{5/7} \gamma \) (\( P < 1.2 \times 10^{-6} \)), percent HbF (\( P < .0035 \)), and absolute HbF (\( P < .00026 \)).

No further division of the total sample into subclasses...
was attempted because the resulting subgroups would have been too small to generate reliable statistical data.

**DISCUSSION**

We have previously established that in African SS patients, the homozygous Senegal haplotype is associated with high mean percent HbF (including a very low incidence of patients with lower than 5% HbF) and fewer dense cells. These findings suggested that the Senegal haplotype could be associated with a benign class of patients, particularly in terms of the degree of hemolysis. In contrast, most patients

(but not all) bearing the Benin haplotype had less than 5% HbF. Other studies of African-Americans, such as those of Hattori et al and more recently Schroeder et al in a larger series, have reported similar results.

SS patients in Africa are generally haplotypically homozygous. In the Americas, because the slave trade gathered victims from all over the African continent, SS patients are generally haplotypically heterozygous. Specifically, most African-American bearers of the Senegal haplotype (the least-represented haplotype associated with HbS) are heterozygous for this character.

Nevertheless, the data presented here demonstrate that African-American SS patients bearing at least one Senegal haplotype have significantly higher Hb levels, reduced reticulocyte counts, and lower bilirubin and LDH levels. In addition, cluster analysis shows that the patients with at least one Senegal haplotype separate themselves from the rest when the Hb, Gγ level, absolute HbF, and percent dense cells (SS4) are taken into account simultaneously. Taken as a whole, these results suggest that this subgroup

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cluster 1</th>
<th>Cluster 2</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dL)</td>
<td>9.6 ± 1.1</td>
<td>8.4 ± 1.4</td>
<td>.004</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>7.5 ± 3.2</td>
<td>11.3 ± 5.8</td>
<td>.02</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>1.9 ± 0.9</td>
<td>3.01 ± 1.6</td>
<td>.01</td>
</tr>
<tr>
<td>Indirect bilirubin (mg/dL)</td>
<td>1.4 ± 0.6</td>
<td>2.58 ± 1.5</td>
<td>.01</td>
</tr>
<tr>
<td>% Gγ</td>
<td>60.3 ± 4.9</td>
<td>46.5 ± 5.6</td>
<td>2.2 × 10⁻¹⁶</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>333.5 ± 89.6</td>
<td>435.1 ± 164.7</td>
<td>.03</td>
</tr>
<tr>
<td>SS4 (% dense cells)</td>
<td>7.8 ± 6.5</td>
<td>14.2 ± 10.1</td>
<td>.03</td>
</tr>
<tr>
<td>% HbF</td>
<td>9.9 ± 5.4</td>
<td>7.4 ± 5.1</td>
<td>NS</td>
</tr>
<tr>
<td>Absolute HbF (g/dL)</td>
<td>0.93 ± 0.47</td>
<td>0.62 ± 0.45</td>
<td>.03</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>91.2 ± 9.2</td>
<td>93.1 ± 11.0</td>
<td>NS</td>
</tr>
</tbody>
</table>

Abbreviation: NS, not significant.
Table 2. Comparison of SS Patients With Either Concomitant \(-\alpha/\alpha\) or \(-\alpha/-\alpha\) in Cluster 1 (at least one Senegal haplotype) With Those in Cluster 2 (other haplotypes)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cluster 1 (mean ± SD)</th>
<th>Cluster 2 (mean ± SD)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dL)</td>
<td>9.9 ± 0.96</td>
<td>8.57 ± 1.18</td>
<td>.01</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>7.35 ± 3.3</td>
<td>10.33 ± 5.06</td>
<td>NS</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>1.82 ± 0.74</td>
<td>2.55 ± 1.90</td>
<td>NS</td>
</tr>
<tr>
<td>Indirect bilirubin (mg/dL)</td>
<td>1.64 ± 0.69</td>
<td>1.93 ± 1.6</td>
<td>NS</td>
</tr>
<tr>
<td>% HbF</td>
<td>60.6 ± 13.9</td>
<td>40.6 ± 6.4</td>
<td>1.2 x 10^-4</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>340 ± 87</td>
<td>390 ± 195</td>
<td>NS</td>
</tr>
<tr>
<td>SS4 (% dense cells)</td>
<td>7.6 ± 8.6</td>
<td>11.9 ± 7.0</td>
<td>NS</td>
</tr>
<tr>
<td>% HbF</td>
<td>12.5 ± 5.4</td>
<td>5.1 ± 4.2</td>
<td>.0035</td>
</tr>
<tr>
<td>Absolute HbF (g/dL)</td>
<td>1.21 ± 0.38</td>
<td>0.43 ± 0.35</td>
<td>.00026</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>90.8 ± 6.4</td>
<td>90.7 ± 9.2</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.97 ± 0.17</td>
<td>0.98 ± 0.40</td>
<td>NS</td>
</tr>
<tr>
<td>Age (y)</td>
<td>36.4 ± 7.6</td>
<td>38.1 ± 10.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

Abbreviation: NS, not significant.

has a milder hemolytic anemia than the Benin, Bantu, and atypical haplotype combinations.

The Senegal haplotype-bearing patients have a significantly higher absolute HbF concentration (and in the case of the SS patients with concomitant \(\alpha\)-thalassemia, also higher percent HbF); hence, it can be tentatively concluded that the effect of the haplotype on the anemia is explained, at least partially, by the differences in HbF associated with these two groups of haplotype combinations, because this Hb variant inhibits Hbs polymerization \(^{16,17}\) and SS cell sickling.\(^8\)

The difference in the incidence of \(\alpha\)-thalassemia incidence between the two groups (cluster 1 and cluster 2) cannot explain the difference observed.\(^8,20\) In a subsample of SS patients with concomitant \(\alpha\)-thalassemia, similar significant differences in Hb level, percent HbF, and absolute HbF, as well as similar trends in the hemolysis related parameters, were observed.

In addition, the clear results obtained with the clustering analysis (that did not include \(\alpha\)-gene status), in which all Senegal haplotype-bearing patients belong to one cluster and all the patients lacking Senegal haplotypes belong to another, strongly argue that the patient's haplotype in combination with the level of Hb, dense cells, and absolute HbF (independent from \(\alpha\)-thalassemia) define two groups of patients that differ in the level of hemolysis.

Another important aspect of Table 1 is that the two groups do not differ significantly in creatinine levels, a parameter that might affect anemia. Worth noting is that the sample presented here corresponds to an adult population with a mean age above 30 years. Caution should be taken in comparing this series with those in which prepubertal or pubescent patients are included, because the level of Hb in those groups might be changing with age.\(^7\)

These results are not in contradiction with the findings of Boyer et al.,\(^7\) which suggest that the HbF level in SS siblings are not fully determined by genetic elements linked to the \(\beta\)-gene cluster. The variance observed around the means HbF clearly suggests that other genetic determinants are involved, including genes not linked to the \(\beta\)-like globin gene cluster.

Moreover, the degree of anemia is most likely multifactorial in SS, and the propensity of the cells to sickle, presumably reduced by the presence of HbF,\(^16,17\) is only one of the factors involved. The degree of marrow expansion, the level of erythropoietin production by the kidneys, and the extent of irreversibly sickled cell formation are individually or in combination participants in determining the level of anemia. These factors, most probably, are under the control of other genetic and nongenetic determinants. Nevertheless, according to the data presented here, the \(\beta\)-gene cluster haplotype is associated with amelioration of the degree of anemia in sickle cell disease.

ACKNOWLEDGMENT

The excellent technical assistance of Fanya Schonbuch and Kathleen Conroy is greatly appreciated. The secretarial assistance of Rose Ann Palestro is gratefully acknowledged.

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